

Page 49, last paragraph

To confirm this was an immunoglobulin light chain variable domain, sequencing was done using the Sequenase 7-Deaza -dGTP DNA Sequencing Kit (USB catalog # 70990) with sequencing primers 607 and GE 108. (See Sequencing Primers in Table 4.) [SEQ ID NOS.: 26-35]

Page 50, first paragraph

A second independent PCR amplification of the light chain from cDNA of primate monoclonal antibody 6G5 was effected using a 5' primer early leader sequence of lambda light chain family 2 (primer 745) and the 3' J region primer 926. (See Primers for PCR of the lambda light chain variable domain of 6G5 in Tables 1-3.) [SEQ ID NOS.: 9-25] The isolated PCR product (see technique above) was cloned into TA vector by using the Original TA Cloning (Kit (Invitrogen Catalog # K2000-01). (The isolated miniprep DNA (see technique above) was examined under agarose gel electrophoresis after digestion with EcoR I restriction endonuclease. The resultant PCR product comprised in the TA vector was then sequenced (as described previously) using Sp6 and M13 (-40) forward primers (See Sequencing primers in Table 4). [SEQ ID NOS.: 26-35] The resultant light chain sequence was identical to that of light chain from the first PCR. This entire sequence [SEQ ID NO.: 1] of the light chain variable domain of primate monoclonal anti-human CD23 antibody 6G5 is presented below.

Page 52, last paragraph – Page 53, first paragraph

The first PCR amplification of the heavy chain variable domain from cDNA of primate monoclonal antibody 6G5 was performed by using the set of early leader sequence primers described supra and the 3' J region primer GE244. These primers are in Tables 1-3 [SEQ ID NOS.: 9-25] infra. This reaction resulted in a 350 base PCR product. This 350 base product (purified as described supra), was digested with Nhe I and Sal I, and ligated into N5LG1 and digested with the same endonucleases in the first PCR amplification. The resultant ligation mixture was transformed into host cells using the same techniques for cloning the light chain. Plasmid N5LG1 containing the 350 base PCR product was then isolated and sequenced (using

sequencing primers 266 and 268). (These Sequencing primers are set forth in Table 4.) [SEQ ID NOS.: 26-35]

---

Page 54, second paragraph

---

A fourth independent PCR was performed using the same primers as the third PCR amplification. This resulted in a PCR product which was isolated and cloned into the TA vector as described previously. The sequence of the fourth independent PCR product was found to be identical to that obtained in the third PCR amplification. This sequence, [SEQ ID NOS.: 3-4] which comprises the heavy chain variable domain of primate monoclonal anti-human CD23 antibody 6G5, is presented below.

---

Page 57, last paragraph – Page 58 continuation of last paragraph on Page 57

---

The first PCR reaction of the light chain variable domain from 5E8 cDNA was carried out using a set of kappa early leader sequence primers and the 3' J region primer GE204. (See primers for PCR of the kappa light chain variable domain of 5E8 in Tables 1-3). [SEQ ID NO.: 9-25] A 420 base PCR product was obtained. The isolated 420 base PCR product was digested with Bgl II and BsiW I restriction endonucleases, cloned into the mammalian expression vector N5KG4P and sequenced using GE108 and 377 primers (which are contained in Table 4). [SEQ ID NO.: 26-35] The mammalian expression vector N5KG4P is identical to the vector N5LG4P except it contains the human kappa light chain constant region in place of the human lambda light chain constant region. Sequencing of this 420 polynucleotide DNA revealed that it contains the entire kappa light chain variable domain.

---

Page 58, last paragraph

---

A second independent PCR of the light chain variable region was performed using the 5' family 1 primer GE201 and the 3' primer Ge204. (See primers for PCR of the kappa light chain variable domain of 5E8 in Tables 1-3). [SEQ ID NO.: 9-25] The isolated PCR product was cloned into the TA vector (using methods previously described) and sequenced using Sp6 and T7 promoter primers. Sequencing revealed that this PCR product was identical to that obtained from the first PCR. The entire sequence [SEQ ID NO.: 3] of the light chain variable domain of primate monoclonal anti-human CD23 antibody 5E8 is presented below.

---

Page 60, last paragraph – Page 61, first paragraph

---

The first PCR of the heavy chain variable domain of 5E8 was performed using a set 5' early leader heavy chain sequence primers and the 3' primer GE210. (See primers for PCR of the heavy chain variable domain of 6G5 and 5E8 in Table 1). [SEQ ID NO.: 9-13] A 420 base PCR product appeared in the family 3 primer reaction. The PCR product was purified and then digested with Nhe and Sal I and cloned into the mammalian expression vector N5KG4P vector (as described previously). The PCR product was sequenced using the 268 and 928 primers. (See sequencing primers in Table 4.) [SEQ ID NOS.: 26-35]

---

Page 61, second paragraph

---

A second independent PCR of the heavy chain variable domain of 5E8 was performed using the family 3 5' primer GE207 and the 3' primer GE210. (See primers for PCR of the heavy chain variable domain of 6G5 and 5E8 in Tables 1-3). [SEQ ID NOS.: 9-25] The isolated PCR product was cloned into a TA vector using the same techniques previously described and sequenced by using Sp6 and T7 primers. Sequencing revealed that the TAC at codon 91 had been changed into TGC.

In order to determine the appropriate codon at 91, a third independent PCR was performed using the same primers as the second PCR (see above). The PCR product was again cloned into a TA vector and sequenced using Sp6 and T7 primers. The sequence was found to be identical to the heavy chain variable sequence obtained in the first PCR. Therefore, the TGC at position 91 in the second independent PCR product is apparently the result of an error introduced during PCR. This entire sequence [SEQ ID NO.: 4] of the heavy chain variable domain of primate monoclonal anti-human CD23 antibody 5E8 is presented below. [SEQ ID NOS.: 7-8]

Page 65, second, third and last paragraphs

---

A first PCR was done using N5KG4P + 5E8 as a template and a 3' primer (corresponding to codon 71 to 79) and which contains a mutation at codon 75 (AAC changed to AAG, Primer MB1654, and a 5' primer at the beginning of the leader sequence (Primer MN1650). (See PCR Primers Used for the Generation of a Glycosylation Mutant of the Heavy Chain Variable Region 5E8 set fourth in Table 5). [SEQ ID NOS.: 36-39]